ANNUAL PROGRESS REPORT

Report Prepared By: Elvin A. Kabat

Date: January 20, 1954
For period: January 1 to December 31, 1953

NR: 121:100

CONTRACT: Nonr 26613

ANNUAL RATE: $13,000. (extended 2 years without additional funds)

CONTRACTOR: Columbia University

PRINCIPAL INVESTIGATOR: Elvin A. Kabat, Ph.D., Professor of Microbiology
Assistants:
Mary E. Carsten, Research Associate in Microbiology (began Sept. 1, 1953)
Joseph M. Schor Graduate Student (Jan. 1 - June 30, 1953)
Peter Allen Graduate student (began Dec.1, 1953)

TITLE OF PROJECT: Immunochemical Criteria of Purity of Proteins and Polysaccharides

Objectives: 1. To develop new immunochemical methods for establishing purity of proteins and polysaccharides. 2. To evaluate limitations or improve existing immunochemical methods. 3. To study the fundamental mechanisms of antigen-antibody reactions.

ABSTRACT OF RESULTS:

a. Since start of project: Mr. Joseph M. Schor carried out a study of the quantitative precipitin curve using three different levels of antibody nitrogen and analyzing precipitates by the microKjeldahl method (range 0.15 to 1.0 mg. N), the Markham Kjeldahl (range 25 - 150 micrograms N) and the Folin-Ciocalteu tyrosine color method (range 10 - 50 micrograms N). His studies showed substantial solubilities of specific precipitates as lower amounts of antibody nitrogen were employed so that large errors in the estimation of total antibody nitrogen content of the sera could result if sera diluted in saline were analyzed. The findings of Maurer that dilution with aged normal serum gave higher values than those with saline or with bovine albumin was confirmed as was the finding that diluting with serum from which the complement had been removed gave values close to those obtained by diluting in saline. These studies are essentially completed and will be prepared for publication.

b. <u>During current report period:</u> While at the University of California on Sabbatical, working with Prof. H. O. L. Fischer and Drs. D. L. MacDonald and C. E. Ballou, the structure of galactinol was established by methylation studies. This work appears in the Journal of the American Chemical Society (1).

Dr. Kabat has carried out a study on the inhibiting effects of various oligosaccharides of known structure on the precipitation of antidextran by dextrans with known numbers of 1 → 6 and 1 → 4 glycosidic linkages which has provided an estimate of the size of the combining site on an antidextran molecule.

Antidextrans with specificities directed toward some multiple of the 1 → 6 glycosidic linkage and other antibodies with non 1 → 6 specificity have been found in sera of humans injected with 1 mg. of dextran (J. Immunol. 1953, 70, 514). Precipitation of antidextran of 1 → 6 specificity by clinical dextran has been found to be inhibited by isomaltotriose, 4- α -isomaltotriosyl-D-glucose and isomaltose; 0.15, 1.0 and 15 μM being required for 50 percent inhibition respectively. Panose was about as effective as isomaltose, isomaltitol was ineffective at 30 μM, some inhibition was obtained with 50 μM of gentiobiose and 110 μM glucose. Maltose type oligosaccharides up to maltopentaose did not inhibit this 1 → 6 system. In addition, an antiserum containing mostly antibody of non 1 → 6 specificity with a small amount of 1 → 6 antibody was studied. Using a dextran NRRL B1299 containing 50 percent each of 1 → 6 and 1 → 4 linkages, it was shown that inhibition of precipitation occurred with 1 → 4 oligosaccharides while 1 → 6 oligosaccharides were much less effective. Maltotriose, maltotetraose and maltopentaose were equally effective and were much better inhibitors than maltose or panose. The extent of residual serum amylase action on maltotetraose and maltopentaose was evaluated and indicated that maltotetraose could possibly be somewhat better as an inhibitor than maltotriose. Some inhibition was obtained with 25 μM of glucose and gentiobiose; cyclooctaamylose and cycloheptaamylose did not inhibit and maltoheptaose was substantially less effective on a molar basis than maltotriose. The data are best interpreted as indicating that each type of antidextran has dimensions complementary to an open chain of at least three α-D-glucopyranose units and probably to part or all of a fourth unit.

The oligosaccharides for this study have been provided by Dr. Allene Jeanes of the Northern Regional Research Laboratory, Dr. M. L. Wolfrom of Ohio State University, Dr. Roy L. Whistler of Purdue University, Dr. Dexter French of Iowa State University, Dr. John H. Pazur of the University of Nebraska and Dr. D. P. Langlois of A. E. Staley and Company.

Dr. Mary E. Carsten joined the group in September 1953 and has been working out technics for measuring the extent of binding of these various anti-dextrans for the various oligosaccharides by equilibrium dialysis. Conditions for ensuring equilibrium have been worked out and the method of Park and Johnson for determining small amounts of reducing sugar has been found satisfactory for the studies. The usual practice of using preservatives in serum has presented difficulties since both the phenol and merthiolate used give substantial blanks in the reducing sugar method. Another difficulty is the necessity for concentrating the human antisera to dextran to give quantities of antibody suitable for equilibrium dialysis studies. Concentration of antibody solutions frequently affects their ability to precipitate with antigen and may create uncertainties as to the amounts of antibody in the solutions. These problems are all being thoroughly investigated.

Mr. Peter Allen who joined the project on December 1, has been investigating the behavior of dextrans with known proportions of $1 \rightarrow 6$, $1 \rightarrow 4$ and $1 \rightarrow 3$ linkages kindly provided by Dr. Allene Jeanes of the Northern Regional Research Laboratory.

PLANS FOR FUTURE:

Immediate: The studies outlined above on oligosaccharide inhibition, equilibrium dialysis, and structural relations among dextrans will be continued. Efforts to concentrate and purify anticextran will be made.

Long range: At the request of ONR in September, the U. S. Public Health Service has been asked to take over this work as of July 1, 1954. The actual application calls for it to be combined with an existing Public Health Grant on Blood Group Substances. Should this not be possible, it is hoped that the ONR will continue to support this work.

REPORTS AND PUBLICATIONS:

1. Kabat, E. A., Macdonald, D. L., Ballou, C. E. and Fischer, H. O. L.: J. Am. Chem. Soc. 1953, 75, 4507

2. Semi-Annual Progress Report #3, January 1 - June 30, 1953
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